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Effect of bupropion on hippocampal neurotransmitters and on peripheral hormonal concentrations in the rat

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Piacentini, M. F., R. Clinckers, R. Meeusen, S. Sarre, G. Ebinger, and Y. Michotte. Effect of bupropion on hippocampal neurotransmitters and on peripheral hormonal concentrations in the rat. *J Appl Physiol* 95: 652–656, 2003. First published April 11, 2003; 10.1152/japplphysiol.01058.2002.—The purpose of the present study was to administer an acute dose of the dual dopamine norepinephrine reuptake blocker bupropion in freely moving rats and to monitor the extracellular neurotransmitter concentrations in the hippocampus via in vivo microdialysis and the peripheral hormonal concentrations via catheterization. A microdialysis probe was inserted in the hippocampus, and samples for serotonin, dopamine, and norepinephrine were collected every 20 min before and after the injection of 17 mg/kg of bupropion, for a total sampling time of 180 min. A catheter was placed in the vena femoralis of the second group of rats, and blood samples were collected before and after bupropion injection for quantification of growth hormone, prolactin, corticosterone, adrenocorticotropin hormone, and β-endorphins. All neurotransmitter levels (dopamine, norepinephrine, and serotonin) significantly increased after bupropion injection. This was accompanied by a significant decrease in prolactin concentrations, whereas the other hormones showed no statistically significant variation. It can, therefore, be concluded that, although bupropion has dual reuptake proprieties, the observed effects both at the central and at the peripheral level seem to be ruled by the dopaminergic system.

dopamine and norepinephrine reuptake inhibitor; hippocampus; hypothalamic-pituitary-adrenal axis; microdialysis; neurotransmitters.

THE ROLE OF NEUROTRANSMITTER release in the regulation of the hormonal response to exercise has drawn the attention of researchers, because of a possible influence on the outcome of performance and fatigue. In our laboratory's previous research, we focused on these integrative mechanisms, using athletes and exercise training as methods to evaluate acute and chronic (physical) stress (12, 13, 16, 18). In our latest study, our laboratory (17) tested the effects of a clinically used dose of bupropion on performance, using the neuroendocrine response to exercise to hypothesize the role of the central neurotransmitter systems.

Catecholamines, in general, are known to play a role in arousal, mood, motivation, vigilance, anxiety, and reward mechanisms and could, therefore, if adversely affected, impair performance (4). Bupropion is a dual dopaminergic and noradrenergic reuptake inhibitor, presenting weak but relatively selective inhibition characteristics of dopamine (DA) reuptake. Its potency as an inhibitor of norepinephrine (NE) reuptake is one-half that for DA, and it shows little affinity for the serotonergic transport system (1). DA is known to have an effect on the release of certain pituitary hormones (6), in particular on prolactin (PRL). Laakman and colleagues (8), however, failed to find an effect of bupropion on PRL and growth hormone (GH) in humans. Therefore, the purpose of the present study was to evaluate the effects of an acute dose of bupropion on the 5-HT, DA, and NE extracellular hippocampal concentrations and on the peripheral hormonal concentrations, to understand the direct effect of this manipulation on brain neurotransmitters and on the hypothalamic-pituitary-adrenal axis (HPA).

METHODS

Animals

Male albino Wistar rats (260–320 g) were used in all experiments. Animals were housed in cages (3 per cage), with food and water available ad libitum. Six rats were used for the microdialysis experiments, and six were used for the catheterization.

The procedures used in this study were carried out according to the European Guidelines on Animal Experimentation and were approved by the ethics committee of the Faculty of Medicine, Vrije Universiteit, Brussel.

Microdialysis Experiments

Surgery and intrahippocampal dialysis. Animals were anesthetized with an intraperitoneal injection of a mixture of diazepam (5 mg/kg) and ketamine HCl (50 mg/kg) and were placed on a stereotaxic frame.

A cannula with a replaceable guide (CMA Microdialysis, Stockholm, Sweden) was implanted through a burr hole in the hippocampus (x: −4.6, y: −5.6, z: +4.6), according to the coordinates described by Paxinos and Watson (15). The can-

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nula was secured to the skull by using dental cement (Durelon Germany). Postoperative analgesia was provided to each rat by giving it a single injection of ketofen (4 mg/kg ip).

Once the animal was in the cage, a microdialysis probe, with a membrane length of 3 mm (CMA Microdialysis), was inserted. The microdialysis probe was connected to a CMA 100 microdialysis pump (CMA Microdialysis) and was perfused with a modified Ringer solution (147 mmol/l NaCl, 4 mmol/l KCl, 2.2 mmol/l $CaCl_2$) at a flow rate of 2 μl/min. Dialysate sampling was started after a minimal period of 12 h after the completion of surgery, permitting the animals to recover sufficiently. During the experiment, dialysates were collected every 20 min from the freely moving animals.

Experimental procedures. After 2 h of baseline collections, animals received an intraperitoneal injection of 17 mg/kg of bupropion (GlaxoSmithKline, Hertfordshire, UK). This particular dosage was chosen because, from previous literature (14, 22), it seems to be a relatively low dose (not in the lowest range) but sufficient to increase central neurotransmitter release.

Sampling continued for 140 min (7 collection samples). Samples were collected and analyzed for 5-HT, DA, and NE. Seventeen milligrams per kilogram of bupropion were considered to be a dose that would have an effect on neurotransmitters and could be comparable to the dose that we gave in humans that was the highest possible without having any side effects. Bupropion was kindly supplied by GlaxoSmithKline.

Chromatographic assays for the determination of NE, DA, and serotonin in hippocampal microdialysates. For the analysis of DA, serotonin, and NE, an off-line microbore liquid chromatography assay (C8, 5 μm; 100 × 1 mm) was used with automatic injection (10 μl) of the samples, as described previously in detail (20). In summary, the assay was based on ion-pair, reversed-phase chromatography, coupled to single-channel electrochemical detection (Decade, Antec, Leiden, The Netherlands). The mobile phase for serotonin and DA consisted of 28 ml acetonitrile and 200 ml of the following aqueous buffer: sodium acetate trihydrate (0.1 M), citric acid monohydrate (20 mM), decane sulfonic acid (2 mM), and sodium EDTA (0.5 mM) adjusted to pH 5.5, whereas the mobile phase for NE consisted of 21–23 ml of acetonitrile and 200 ml of the previously described buffer. The flow rate through the column was 90 μl/min. Because of the high pH 5.5 of the mobile phase, a low-oxidation potential was set (+450 mV vs. Ag-AgCl). The retention times for NE, DA, and serotonin were 3, 7, and 14 min, respectively, with a quantification limit for all compounds of 20–40 pM.

Catheterization Experiments

Surgical intervention. Animals were anesthetized with an intraperitoneal injection of a mixture of diazepam (5 mg/kg) and ketamine HCl (50 mg/kg).

First, a heparin-coated polyurethane catheter (Solomon Scientific, Harleysville, PA) was tunneled subcutaneously and exited at the back of the neck. Then the catheter was implanted in the femoral vein and continuously perfused with saline (flow rate: 0.05 ml/min). The catheter was used for blood sample collection (1 ml).

Experimental procedure. After the surgery, rats were placed in the cage, and the start of the experiment was ~1 h after full awakening. A 1-ml blood sample was taken at baseline and 20 min after bupropion injection (17 mg/kg ip), and two more samples were taken, at 40-min time intervals. After each sample, the same volume of saline was injected. The catheter was attached to saline enriched with 1 ml of heparin (LEO Pharma, Zaventem, Belgium) and rinsed the catheter with a constant flow.

Analytic procedure for the determination of the hormones in the rats plasma. Blood samples for the quantification of ACTH and β-endorphins (β-E) were collected in prefrozen 4.5-ml K_3-EDTA vacutainer tubes (Beckton Dickinson Vacutainer System Europe, Belliver Industrial Estate, Plymouth, UK), immediately centrifuged at 3,000 rpm (Labofuge, Heraeus Christ, Van Der Heyden, Brussels, Belgium) for 10 min, and frozen at −20°C until further analysis. Blood samples for quantification of cortisosterone, GH, and PRL were collected in 8.5-ml vacutainer serum tubes (Beckton Dickinsons Vacutainer System Europe, Belliver Industrial Estate) and were kept at room temperature until clotting before centrifuging at 3,000 rpm (Labofuge, Heraeus Christ, Van Der Heyden) for 10 min.

Samples were then assayed via RIA for ACTH and β-E (Phoenix Peptide, Belmont, CA), PRL (DRG Instruments, Marburg, Germany), GH (Linco Research, St. Charles, MO), and cortisol (DSL, Sinsheim, Germany).

Statistical Analysis

Data from the microdialyis experiments are presented as means ± SE (in nM). The six baseline collections (preinjection collections) were averaged, and all data were analyzed by one-way ANOVA for repeated measures. If the overall *F*-test showed significance, then post hoc analysis was performed by using the Fisher's paired least significant difference (PLSD) test to evaluate statistical significance. Significance was set at $P < 0.05$. Hormonal data are presented as means ± SE. Comparisons with baseline were analyzed by one-way ANOVA for repeated measures. If the overall *F*-test showed significance, then post hoc analysis was performed by using the Fisher's PLSD test. Significance was set at $P < 0.05$.

RESULTS

Effects of Bupropion on Extracellular NE, 5-HT, and DA Concentrations in the Hippocampus of Freely Moving Rats

Extracellular DA levels increased significantly (3.5-fold), reaching their highest value 40 min postinjection (0.10 ± 0.01 to 0.37 ± 0.10 nM) (Fig. 1*B*). These DA concentrations remained significantly elevated until 60 min postinjection. After that, the levels decreased, but they did not return to baseline values 2.5 h postinjection.

Similar to that observed for DA, extracellular NE levels increased ~3.5-fold after bupropion injection (Fig. 1*A*). They remained elevated until 120 min postinjection. The peak concentration for NE was observed after 40 min: they increased from 0.16 ± 0.03 to 0.40 ± 0.03 nM. Afterward, the NE levels started to decrease, but they did not return to baseline levels by the end of the experiment.

5-HT showed no significant change after bupropion injection (Fig. 1*C*).

Effects of Bupropion on Peripheral Hormonal Concentrations

PRL plasma concentrations decreased and reached a 75% decrease 100 min postinjection (from 23.5 ± 7.2 to

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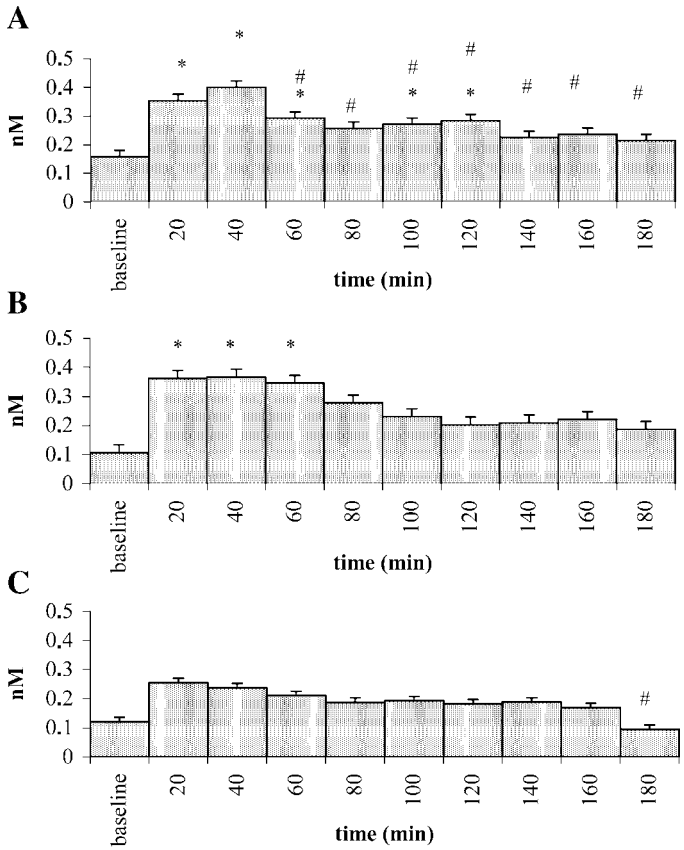

Fig. 1. Effect of bupropion (17 mg/kg ip) on extracellular norepinephrine (*A*), dopamine (*B*), and serotonin (*C*) concentrations in the hippocampus of the freely moving rat. Microdialysis samples were collected every 20 min. Data are means ± SE in nM ($n = 6$) and were analyzed by one-way ANOVA for repeated measures followed by Fisher's paired least significant difference post hoc test ($\alpha = 0.05$). Statistically significantly different from *baseline values and #40-min time point, $P < 0.05$.

5.7 ± 4.1 ng/ml) (Fig. 2*A*). All of the other hormones showed no significant change after bupropion injection.

DISCUSSION

The importance of linking central and peripheral measurements derives from the increasing interest in understanding the specific effect of neurotransmitters on the outcome of performance. To our knowledge, this is the first study to evaluate the peripheral and central effects of bupropion in freely moving animals.

The effects of bupropion on the HPA axis have been previously studied only in humans (8, 17), and neither study found an effect on PRL and GH. The purpose of the present study was to evaluate whether bupropion, a dual dopaminergic noradrenergic reuptake inhibitor, would increase hippocampal NE and DA extracellular concentrations and to evaluate whether the reuptake properties of the drug were associated with peripheral hormonal concentrations.

Bupropion is a weak, but relatively selective, inhibitor of DA reuptake. Its potency as an inhibitor of NE reuptake is one-half of that of DA, and it shows little affinity for the serotonergic transport system (1). The

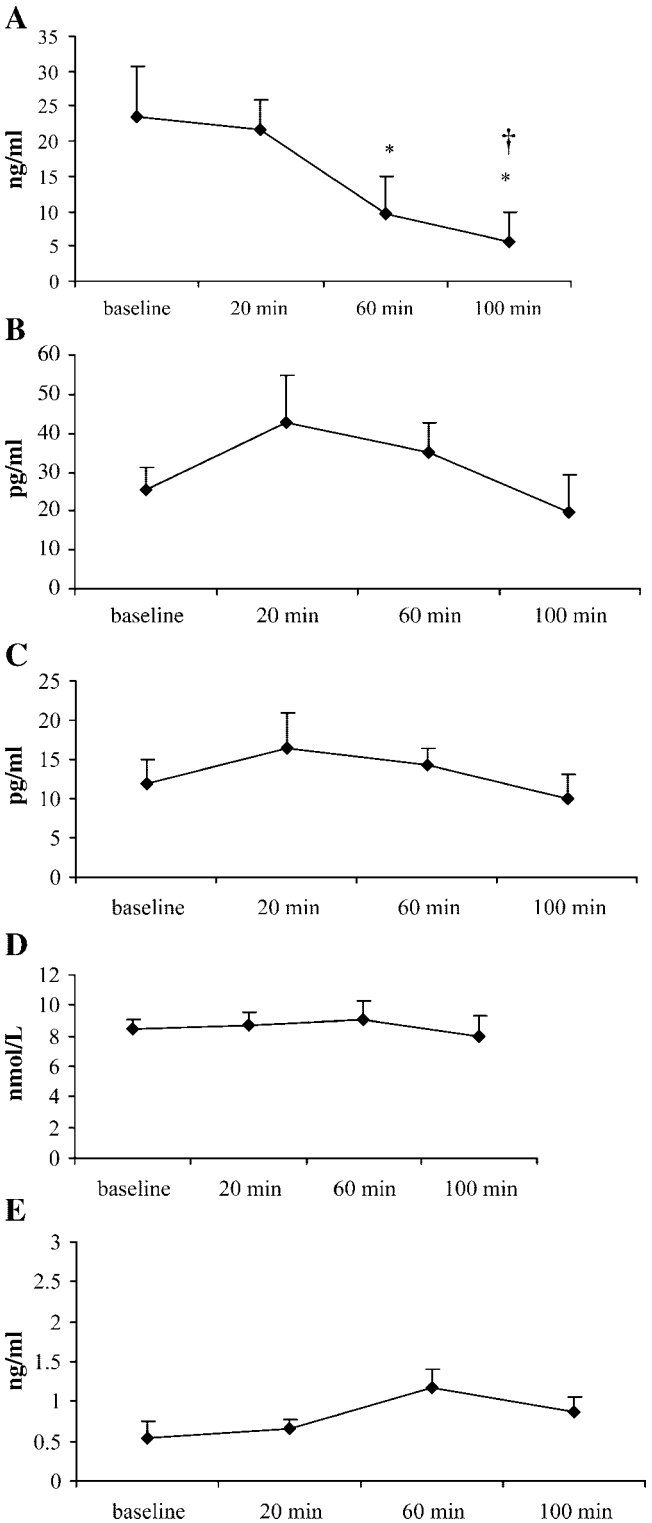

Fig. 2. Effect of bupropion (17 mg/kg ip) on plasma concentrations of prolactin (*A*), ACTH (*B*), β-endorphines (*C*), corticosterone (*D*), and growth hormone (*E*) in freely moving rats. Data are means ± SE ($n = 6$) and were analyzed by one-way ANOVA for repeated measures followed by Fisher's paired least significant difference post hoc test ($\alpha = 0.05$). Statistically significantly different from *baseline values and †20-min time point, $P < 0.05$.

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major metabolites of bupropion, hydroxybupropion, and threohydrobupropion are weaker inhibitors of DA, 5-HT, and NE reuptake (1). Although bupropion is a more potent inhibitor of DA reuptake than other antidepressants, its metabolites are not (1).

Microdialysis

The choice of the hippocampus as an area of interest relates to the fact that there is a mutual influence of the hippocampus on the output of hormones and on the effect of the same hormones on hippocampal function. The principal circulating glucocorticoid in rodent, corticosterone (cortisol in human), targets receptors throughout the body, but also in the brain, and, in particular, the hippocampus has plenty of glucocorticoid and mineralcorticoid receptors (9). There is, therefore, evidence for a modulation of hippocampal function by hormones, as well as a direct control of the hippocampus on the HPA axis and the production of downstream hormones, including, but not restricted to, adrenal glucocorticoids and catecholamines (for review, see Ref. 9).

The results of the present study demonstrate that an acute dose of bupropion increases the extracellular concentrations of DA and NE in the hippocampus of freely moving rats with no effect on 5-HT. Both microdialysis and electrophysiological studies show that acute doses of bupropion have an effect on DA and NE in the striatum and nucleus accumbens in a dose-dependent manner (3, 14). Bupropion, but not its metabolites, reduced the firing rates of dopaminergic neurons in a dose-dependent manner (5). It is evident from the present and the previous animal studies that bupropion is able to increase DA and NE in different brain regions.

Hormonal Results

As expected, PRL concentrations decreased immediately after injection, reaching a 75% decrease 100 min after injection. It is now well established that the release of PRL is tonically inhibited by central mechanisms in which DA is involved (2). However, NE, via activation of the α_2-receptors, has an inhibitory influence on PRL release as well (7). This result is confirmed by a previous study (21) that measured GH and PRL concentrations in humans and in rats after an acute bupropion injection. The authors (21) found, as we did, a decrease in PRL and no effect on GH concentrations. Human studies failed to find a decrease in PRL concentrations after different doses of bupropion (8, 17), as expected by a dopaminergic reuptake inhibitor. The pharmacological profile of bupropion, however, is different in humans and in animals due to the fact that rodents lack the metabolite hydroxybupropion (3). In humans, the effects of bupropion may result from the large concentration of the metabolite, which has mainly noradrenergic effects (3), and this would confirm the different hormonal response between humans and animals.

DA is known to have effects on PRL and GH, but has no effects on ACTH and cortisol (2), or could even be involved in mechanisms that inhibit ACTH release (2). In the present study, in fact, ACTH and β-E were not affected by bupropion, most probably due to the inhibitory role of DA on the release of the pituitary hormones (6).

Peripheral Hormone Concentrations as Markers for Central Effects

The combination of these central and peripheral data are important for researchers interested in the role of neurotransmitters on performance and fatigue. In particular, the hippocampus itself has an important role on stress reactivity (10). If adrenal steroids typically have adaptive effects in the short run, they promote pathophysiology when there is either repeated stress or deregulation of the HPA axis, and the feedback mechanisms to the hippocampus may be deregulated (11).

PRL has been considered the peripheral marker of serotonergic function, but it might be that, when the DA influence, e.g., from hippocampus, is present (as in this experiment), the reaction is more complex. Similarly, by supplementing humans with fluoxetine, a 5-HT reuptake inhibitor, (12), the PRL increase due to the reuptake inhibitor during exercise was lower compared with that during the placebo trial. This could again indicate that there was a dopaminergic increase secondary to the drug-induced 5-HT increase (19) that influenced the PRL response to exercise. It seems, according to the present and past results, that PRL can be considered a better marker for dopaminergic than for serotonergic central activity.

In summary, the weak but relatively selective inhibition of DA reuptake due to bupropion is confirmed by the present microdialysis data: a significant increase in hippocampal DA and NE concentrations. The peripheral hormonal output seems to be regulated more by the dopaminergic system, due to the fact that we found a decrease in PRL and no effect on the other hormones that would have increased under the noradrenergic effect alone.

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DISCLOSURES

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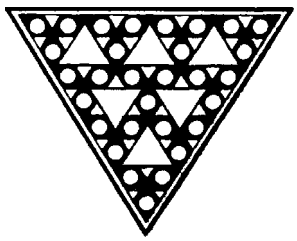

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